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biologic sensitizers results in decreasing or completely inhibiting toxic action, but no protection was afforded by the addition of glucose, starch or gelatin. This interesting observation appeared to us to demand further consideration, since there are definite chemical differences between the inhibiting and noninhibiting substances. The inhibitory effect cannot be attributed to the amphoteric character or the colloidal nature of the protective substances. There is a striking difference in the nature and content of the amino acids of gelatin and egg albumin. The former wholly lacks tyrosin, tryptophan and cystin, and contains only minimal amounts of alanin, serin, aspartic acid, phenylalanin and histidin.[6]

Former work [7] on the protection afforded to immune bodies against the toxic action of ultraviolet light showed that the aromatic amino acids have the power of absorbing these waves, and since action by light cannot take place without absorption, the presence of these amino acids in the protein molecule is essential for this reaction. In the present work we have sought to find a correlation between the protection afforded to red cells by certain proteins against lysis by eosin and the nature of the amino acids in the molecule and our results indicate that the presence of tyrosin or tryptophan determines ability to protect.

The experiments were carried out with eosin (Grübler's w. g.) and red blood cells were used to indicate toxic action; 0.5 c c of a 5% saline suspension of red cells (sheep or ox) was placed in each of a number of small test tubes and to each, 1 c c of a 1 : 10,000 eosin in salt solution was added. The substances to be tested for protective action were likewise made up in salt solution in the concentrations as given in the table and the reaction adjusted to P_H 7.5-8.0. The tubes containing the suspension of red cells, eosin and substance to be tested for inhibitory action and also a number of control tubes were placed in the sunlight for a period of 30 minutes and after exposure immediately placed in the ice chest. The tubes were inspected at the end of 3 hours and again after 18 hours to determine the amount of lysis. Only in those tubes which after 3 hours showed that some hemolysis had taken place, was a slight increase noted after 18 hours. A certain number of tubes similar to the above kept in the dark in order to rule out the possibility of any unlooked for factor being concerned in the reaction.

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[6] Fischer, Levene and Aders: Ztschr. physiol. Chem., 1902, 35, p. 70; Levene and Beatty: Ztschr. physiol. Chem., 1906, 49, p. 252.

[7] Harris and Hoyt: Science, 1917, 46, p. 318; University of Cal. Pub. Path., 1919, 2, p. 245; Hill and Schmidt: J. Infect. Dis., 1919, 25, p. 335.

From the table it will be noted that protein substances, other than egg-white and blood serum, namely, ovomucoid, casein, edestin, Witte's peptone and deutero-albumose, inhibit the lytic action of eosin on red cells. Casein lacks glycocoll, gelatin contains about 16%; apparently the presence or absence of this amino acid in the protein molecule is immaterial. The experiments with pure amino acids show definite results. Of those tested, glycocoll, leucin, aspartic acid, α and β alanin, glutamic acid, cystin, phenylalanin and taurin offer no protection while tyrosin and tryptophan are very effective. To a limited extent this coincides with results reported by Hill and Schmidt[7] on the protection afforded by amino acids to immune bodies against the toxic action of ultraviolet light. There is, however, a striking difference, illustrated in the instance of phenylalanin. The inability of this amino acid to protect against eosin hemolysis shows that the benzene ring alone is not the determining factor. The lack of protection shown by mandelic acid, hydrocinnamic acid and phenylpropionic acid supports this view. Tyrosin, despite its comparatitve insolubility (1:2,400), and tryptophan[8] offer marked protection to red cells. The hydroxyphenyl group in a molecule is one of the factors determining ability to protect. This is clearly shown by the fact that hydrocinnamic acid and mandelic acid offer no protection while both orthocumaric and orthohydrocumaric exhibit marked protective ability. Addition of di- and trioxybenzoic acid, resorcin, salicylic acid, pyrogallol or phenol to a mixture of red cells and eosin solution prevents hemolysis on exposure to light.

Since gelatin contains appreciable quantities of prolin (5%), arginin (7.6%), lysin (2.8%) and oxyprolin (6.4%), these amino acids appear to play no part in protecting red cells against the photodynamic effect of eosin. Valin, isoleucin, serin and histidin were not available and no results can be reported. In view of the structure of these amino acids, it appears probable that they are also without influence. The first three do not have a ring structure and histidin lacks the benzene ring. Little or no protection is afforded by inosite. It must be remembered that although there are six hydroxy groups in this molecule, the substance is a derivative of the reduced benzene ring, hexamethylene, and does not contain a hydroxyphenyl group.

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[8] This preparation was recrystallized repeatedly and did not give the Millon test.

The protection afforded to red cells against eosin hemolysis is not due to the absorption of those light waves that cause eosin to fluoresce. It was found that tubes containing red blood cells and eosin surrounded by a solution of a "protective substance" are hemolyzed in the same time as those surrounded by a water jacket. Tubes containing cells, eosin and a protective substance fluoresce strongly when placed in sunlight; the function of the protective agent does not consist in the absorption of all of the visible fluorescent waves. We have confirmed previous observations that the eosin must be in intimate contact with red blood cells in order to obtain hemolysis. No hemolysis was observed when a suspension of red cells in a quartz test tube was placed in a solution of eosin and exposed to sunlight. Change of acidity is also not a factor concerned in the reaction since no inhibition of hemolysis was observed on addition of a neutral phosphate buffer mixture.

Since photochemical action is conditioned on absorption of light waves, it appears to us that the protection afforded by certain substances is connected with their ability to absorb the active rays and thus prevent their absorption by the red blood cells. If this hypothesis is correct, the toxic action of eosin on protoplasm is determined by the presence of tyrosin or tryptophan in the protein molecules of the cell.

Although our results have shown that certain relations exist between the structure of the protein molecule and ability to protect against the photodynamic effect of eosin, they must nevertheless be considered largely of a qualitative nature. Although certain substances protect red cells against eosin hemolysis, the action is largely dependent on the concentration of eosin, time of exposure and concentration of the protective substance. A number of experiments were also carried out with rose bengal (1:200,000) as photosensitizer. The results obtained were less pronounced than in the eosin series, due no doubt, to the greater potency of this dye in causing hemolysis.

It may be of interest to mention that Bovie[9] has recently found that in a solution of eosin which had been in contact with thymol for some time and exposed to diffuse daylight, a precipitate in the form of a surface film appeared, indicating that a reaction had taken place between these substances.

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[9] Chem. Engineer, 1919, 27, p. 141.

THE EFFECT OF THE ADDITION OF CERTAIN SUBSTANCES ON THE HEMOLYSIS OF RED CELLS BY EOSIN

Substance Added	Formula	Concentration	Result on Hemolysis
(a) Proteins:			
Gelatin	....	1%	No inhibition
Blood serum	....	1:5	Complete inhibition
Casein	....	1%	Complete inhibition
Edestin	....	1%	Complete inhibition
Witte's peptone	....	1%	Complete inhibition
Ovomucoid	....	1%	Complete inhibition
Deuteroalbumose	....	1%	Complete inhibition
(b) Amino acids:			
Glycocoll	$CH_2NH_2.COOH$	1%	No inhibition
Leucin	$(CH_3)_2:CH.CH_2.CH:(NH_2).COOH$	1%	No inhibition
Alanin	$CH_3.CH(NH_2).COOH$	1%	No inhibition
Alanin	$NH_2CH_2.CH_2.COOH$	1%	No inhibition
Aspartic acid	$HOOC.CH_2.CH(NH_2),COOH$	1%	No inhibition
Glutamic acid	$HOOC.CH_2.CH_2.CH(NH_2).COOH$	1%	No inhibition
Taurin	$NH_2.CH_2.CH_2.SO_3H$	1%	No inhibition
Cystin	$HOOC.CH(NH_2)CH_2.S$— $S.CH_2.(NH_2)CH.COOH$	Saturated solution	No inhibition
Phenylalanin	$C_6H_5.CH_2.CH(NH_2).COOH$	1%	No inhibition
Tyrosin	$OH.C_6H_4.CH_2.CH(NH_2).COOH$	Saturated solution	Complete inhibition
Tryptophan (3 spec.)	$C_8H_6N.CH_2.CN(NH_2).COOH$	M/40	Complete inhibition
(c) Other substances:			
Mandelic acid	$C_6H_5.CHOH.COOH$	1%	No inhibition
Hydrocinnamic acid	$C_6H_5.CH_2.CH_2.COOH$	1%	No inhibition
Phenlypropionic acid	$C_6H_5.C \vdots C.COOH$	1%	No inhibition
Inosite	$C_6H_6(OH)_6$	M/40 M/200	Slight inhibition No inhibition
Ortho-cumaric acid	$OH.C_6H_4.CH.CH.COOH$	M/40 M/200	Complete inhibition Partial hemolysis
Ortho-hydrocumaric acid	$OH.C_6H_4.CH_2.CH_2.COOH$	M/40	Partial hemolysis
Gallic acid	$C_6H_2.(OH)_3.COOH$ 3:4:5	M/40 M/200	Complete inhibition Complete inhibition
Pyrogallol	$C_6H_3.(OH)_3$ 1:2:3	M/200 M/1000	Complete inhibition Partial hemolysis
Trioxybenzoic acid	$C_6H_2(OH)_3.COOH$ 2:3:4	M/200 M/1000	Complete inhibition Partial hemolysis
Dioxybenzoic acid	$C_6H_3.(OH)_2COOH$ 2 : 5	M/40 M/200	Complete inhibition Partial hemolysis
Thymol	$C_6H_3(CH_3).OH.CH(CH_3)_2$ 1:3:4	Saturated solution	Partial hemolysis
Phenol	$C_6H_5.OH$	M/40	Partial hemolysis
Sodium benzoate	$C_6H_5.COONa$	1%	No inhibition
Salol	$C_6H_4.(OH)COOC_6H_5$ 1:2	Saturated solution 1:1000	No inhibition
Glucose	$OHCH_2.(CHOH)_4.CHO$	1%	No inhibition
Cholesterol	$C_{27}H_{43}OH$	0.3% suspension in 1% gelatin	No inhibition
Resorcin	$C_6H_4.(OH)_2$ 1:3	M/200 M/1000	Complete inhibition Partial hemolysis
Salicylic acid	$C_6H_4.(OH).COOH$	M/40	Partial hemolysis

SUMMARY

The object of the investigation was to determine the cause of the inability of gelatin to protect red cells against the photodynamic action of eosin. Since gelatin lacks certain amino acids that are contained in the protein molecules which afford protection, experiments were

carried out to determine the protection afforded by individual amino acids.

It was found that tyrosin and tryptophan offer marked protection, while phenylalanin, glycocoll, leucin, aspartic acid, alanin, cystin and glutamic acid are ineffective. Certain other substances containing a hydroxyphenyl group in the molecule also protect red blood cells against hemolysis by eosin.

Since action by light cannot take place without absorption, it is possible that the protection afforded to red cells by certain substances against the photodynamic effect of eosin is due to the absorption of the active rays by the protective substance.